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EXAMINER	
MYERS, CARLA J	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mailroom@bskb.com

Office Action Summary	Application No.	Applicant(s)	
	10/695,744	PATERLINI-BRECHOT, PATRIZIA	
	Examiner	Art Unit	
	Carla Myers	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 August 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,4,5,9,11-18,20-22,25,30 and 31* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,4,5,9,11-18,20-22,25,30 and 31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *2/20/08*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on August 5, 2008 has been entered.

2. Applicant's arguments and amendments set forth in the response of August 5, 2008 have been fully considered but are not persuasive to place all claims in condition for allowance. The previous rejections under 35 USC 103 have been obviated by the amendments to the claims. However, this action contains new grounds of rejection which address the claims as amended. This action is made non-final.

3. Claims 1, 4, 5, 9, 11-18, 20-22, 25, 30 and 31 are pending and have been examined herein.

Claim Rejections - 35 USC § 112

4. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 4, 5, 9, 11-18, 20-22, 25, 30 and 31 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a

way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

The specification as originally filed does not appear to provide support for the amendment to the claims to recite "an applied filtration depresssure, said filtration depressure being in the range of 0.05 to 0.8 bars." The response points to page 19, lines 1-2 as providing support for this amendment. However, the cited portion of the specification teaches only that the filtration pressure applied is from 0.05 to 0.8 bars. The specification does not provide support for the concept of providing a filtration depressure.

Regarding claim 30, the specification as originally filed does not appear to provide support for the amendment to recite "a pore density of 1.10^5 pores/cm2." The response points to page 9, lines 14-15 as providing support for this amendment. However, the cited portion of the specification teaches filters having a pore size of 1×10^5 pores/cm^2.

Claim Rejections - 35 USC § 112 second paragraph

5. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 4, 5, 9, 11-18, 20-22, 25, 30 and 31 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1, 4, 5, 9, 11-18, 20-22, 25, 30 and 31 are indefinite over the recitation of "applied filtration depressure." This phrase is not defined in the specification or claims and there does not appear to be any art recognized definition for this phrase. Accordingly, one of skill in the art cannot determine the meets and bounds of the claimed subject matter.

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 4, 5, 9, 11, 12, 20-22, 25 and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kalionis in view of Vona (American Journal of Pathology. January 2000. 156: 57-63; cited in the IDS), and further in view of Bisconte (FR 2782730; cited in the IDS of 2/12/04; note that the English translation of this document is US 2002/0028431; see page 1 of the FR 272730 document as filed).

Kalionis teaches a method for prenatal diagnosis of fetal cells isolated from maternal blood. The reference (page 3) states that "(t)he present invention is directed to a method for easily enriching and identifying trophoblast cells in maternal peripheral blood in the presence of a population of blood cell types. The enrichment, identification and analysis of trophoblast cells in peripheral blood provides a means by which non-invasive prenatal diagnosis can be carried out. This method is therefore of particular

value in prenatal testing to obtain genetic and/or biochemical information about the fetus."

The method of Kalionis (pages 5-7) comprises the steps of:

a) diluting a sample of maternal blood in a solution comprising a reagent for lysing red blood cells;

b) filtering the diluted sample of maternal blood through a filter according to size, in order to separate fetal cells from maternal blood cells;

c) analyzing the cells retained on the filter by immunostaining for trophoblast-specific markers, in order to confirm the identify of the cells as being of fetal origin (see also page 8);

d) analyzing individual cells by in situ hybridization and immunostaining to demonstrate that the cells are fetal cells (see also pages 10 and 18); and

e) analyzing the individual fetal cells to detect a genetic anomaly or to determine the sex of the fetal cells (see also pages 9-10 and page 21).

Kalionis does not teach collecting the individual fetal cells retained on the filter by microdissection, wherein the microdissection uses a laser to recover single collected cells in a tube, and analyzing the isolated fetal cells by lysing the cells, pre-amplifying the cells and using the preamplification product to demonstrate the fetal origin of the isolated cells and to carry out prenatal diagnosis).

However, Vona (pages 58-60) teaches methods for isolating rare cells from blood wherein the methods comprise:

a) passing a blood sample through a filter to retain target cells according to size;

b) analyzing the cells retained on the filter to confirm their identity by hematosylin-eosin staining or by immunostaining with antibodies to specifically detect tumor cells;

c) using microdissection with the aid of a laser to individually collect the isolated cells retained on the filter into a tube in order to obtain a single collected cell;

d) lysing the single collected cell whereby the genome of the single cell is made accessible to amplification primers;

e) amplifying the genome of the lysed single cell to obtain a pre-amplification product; and

f) using one–fifth (i.e., 5 out of 60 µl) of the preamplified DNA preparation to amplify the pre-amplification product, to thereby analyze the isolated DNA from the single lysed cell.

Vona states that the use of microdissection to isolate individual cells, followed by the amplified of DNA from the individual cells provides the advantage of a highly sensitive technique for detecting genetic abnormalities (page 58). It is also stated that the method of isolating cells by filtration followed by amplification of the nucleic acids in the isolated cells provided improved results over methods which relied on PCR alone (pages 58 and 62). The method is characterized as being "easy to perform, rapid, and inexpensive" (page 61). The method also provides the advantage of allowing for the isolation of individual cells without damaging the morphology of the cells, thereby providing increased sensitivity (page 61). Additionally, Vona (page 62, col. 2) states that the method "allows the isolation of large, circulating, nontumorous cells. For example,

the isolation of trophoblastic cells from the peripheral blood of pregnant women has been initiated in our laboratory and may constitute an important step toward improving the prenatal diagnosis of genetic diseases."

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the prenatal diagnosis method of Kalionis so as to have applied the methodology disclosed by Vona of filtering the blood sample to obtain isolated rare (fetal) cells, analyzing the individual cells by morphological staining or immunological staining, and collecting the individual fetal cells retained on the filter by laser microdissection in order to have provided an efficient and effective means for isolating the individual fetal cells that would allow for the confirmation of the identity of the individual cells and the genetic analysis of the individual cells. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have preamplified the genetic material obtained from the isolated cells prior to performing amplification reactions to analyze the DNA from the individual cells, as set forth by Vona, in order to have achieved the benefit of increasing the sensitivity of detection of genetic anomalies in the isolated cells and confirming the fetal origin of the isolated cells.

Regarding step a) of the present invention, Kalionis (page 7) teaches that prior to filtration, the maternal blood may be diluted and treated by a variety of techniques that will lead to the lysis of erythrocytes (red blood cells). In particular, Kalionis teaches that dilution of the blood sample in a hypotonic buffer results in the lysis of erythrocytes (red blood cells), thereby reducing the number of cells needed to be filtered and reducing the

incidence of coagulation. Accordingly, Kalionis teaches the step of diluting a sample of maternal blood in a filtration solution comprising a reagent for lysing red blood cells.

Furthermore, Vona teaches that prior to filtration, the blood sample is diluted 1:10 in a solution containing saponin (an agent for lysing red blood cells) and paraformaldehyde (a reagent for fixing nucleated cells). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kalionis so as to have diluted the maternal blood in a buffer that contained both an agent for fixing nucleated cells and an agent for lysing red blood cells in order to have provided an effective means for preparing the blood sample for filtration and for further analysis of nucleated cells present in the blood sample.

Regarding step b), Kalionis (page 4) teaches filtering the maternal blood through a filter having a pore size of 10 µm. Kalionis does not teach filtering the blood sample through a polycarbonate membrane with a pore size of 8 µm, and a pore density in the range of 5×10^4 to 5×10^5 pores/cm^2 and wherein a filtration pressure of 0.05 to 0.8 bars is applied to the filter. does not specifically teach pore sizes of 8 µm. However, Vona (page 58) teaches that the blood samples are filtered through a polycarbonate filter calibrated with 8 µm cylindrical pores. Vona also teaches that each sample is filtered through a 0.6-cm diameter circular spot on the filter and that the cells were laser cut from the filter for collection.

Moreover, Bisconte (FR 272730; citations are with respect to the English translation of this document – 2002/0028431) teaches a filtration device for isolating

rare cells, including epithelial cells from blood (para [0013-0016]. The filtration device has a pore size of approximately 8 μm (para [0020]).

While the combined references do not teach that the membrane has a pore density of 5 X 10^4 to 5 X 10^5 pores/cm^2, to have determined the optimum density of the pores that would allow for the isolation and collection of single cells from the filter membrane would have been obvious to one of ordinary skill in the art and well within the skill of the art. As discussed in MPEP2144.05(b), "(w)here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation. In re Aller, 220 F.2d 454, 105 USPQ 233, 235 (CCPA 1955). In particular, Bisconte (para [0039] and Vona (page 58, col. 1) each teach the criticality of selecting a filter wherein the pore size is sufficient to retain the cell of interest and wherein the filter allows for the separation and isolation of individual cells. Accordingly, the selection of a polycarbonate filter having an optimum pore density, including a pore density of 5 X 10^4 to 5 X 10^5 pores/cm^2, would have been obvious to one of ordinary skill in the art and well within the skill of the art at the time the invention was made in order to have to have accomplished the objective of isolating and collecting the single fetal cells, thereby facilitating the method of prenatal diagnosis.

Regarding the recitation of an applied pressure of 0.05 to 0.8 bars, Bisconte (para [0037]) teaches the use of the filtration device to isolate rare pathogenic cells from blood samples, wherein the filtration device has a partial vacuum of approximately 50,000 PA (i.e., 0.5 bars) pressure applied under the filter. Further, the selection of an optimal filtration pressure based on the type and size of cell to be isolated was well

within the skill of the art at the time the invention was made. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have further modified the method of Kalionis so as to have used a filtration pressure of 50,000 PA in order to have accomplished the objective set forth by Bisconte (FR 272730) of providing an effective method for isolating and collecting rare cells from blood samples in a manner sufficient to maintain the integrity of the cell and to have allowed for the isolation of individual cells.

Regarding step c), Kalionis teaches that fetal trophoblast cells can be identified and distinguished from maternal cells by staining with trophoblast-specific antibodies (pages 5 and 8). Vona teaches that in the method to isolate rare cells, the cells retained on the filter are analyzed for their morphological properties by hematoxylin-eosin staining (page 59) and can be identified as tumor cells by immunostaining. In the method of Vona, the immunostaining occurs while the cells are retained on the filter. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have analyzed the fetal cells retained on the filter by immunostaining with trophoblast-specific antibodies in order to have confirmed the identity of the cell as being a fetal cell prior to microdissection and isolation of the fetal cell from the filter.

Regarding step f), Kalionis teaches analysis of the individual cells to ensure that the cells are of fetal origin. It is stated that many mRNAs are trophoblast specific and are not present in maternal cells and thereby these mRNAs can be detected as indicative of a fetal cell (page 10). The presence of the Y chromosome can also be

detected as indicative of the identity of the fetal cell (pages 22-23). Kalionis further teaches analyzing the individual fetal cells for a genetic abnormality (page 5). Vona teaches that the isolated cells are further analyzed by PEP PCR and exemplifies methods wherein two distinct amplification products are produced - p53 and HLA amplification products, thereby allowing for the analysis of "two clinical features." Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have analyzed the trophoblast cells isolated from maternal blood by performing a pre-amplification reaction, and then analyzing the pre-amplification products by PCR methods in order to both verify the fetal origin of the isolated single cells and to carry out prenatal diagnosis.

With respect to claims 4 and 5, in the method of Vona, the cells retained on the filter are collected individually by microdissection, wherein microdissection consists of laser cutting a portion of the filter on which the cells are retained and recovering a single collected cell in a suitable tube (pages 58-60).

With respect to claims 9 and 11, modification of the method of Kalionis to collect the cells on the filter by microdissection and to preamplify the nucleic acids present in the collected cells prior to analysis would have resulted in a method of identifying one or more genetic targets, and particularly a genetic or chromosomal anomaly, in the preamplification product.

Regarding claims 12, in the method of Vona (see page 60), the amplification is performed using less than one fifth of the preamplification product - i.e., 5 out of 60 µl of the extension product.

With respect to claim 20, the reference teaches that the maternal blood samples are obtained form women at 30-37 weeks of pregnancy (see Table 1).

With respect to claim 21, the reference (page 7) teaches obtaining and filtering 5-100 ml of maternal blood. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have practiced the method of Kalionis in view of Vona using a sample of about 5ml and then further diluting the sample since this constitutes an acceptable quantity of maternal blood to obtain from a pregnant woman in order to allow for the analysis of fetal cells present in the maternal blood.

With respect to claim 22, Kalionis teaches that the blood can be diluted with an isotonic buffer to reduce the viscosity prior to filtering or in a hypotonic buffer to lyse red blood cells and thereby reduce the number of cells that need to be filtered and the incidence of coagulation. Kalionis does not specifically exemplify methods in which the blood is diluted 10 to 100 fold. However, Vona (page 58) teaches collecting 6 ml of blood and diluting the blood 1:10 in filtration solution prior to filtering. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kalionis so as to have diluted the blood 1:10 fold in the filtration solution prior to filtering in order to have reduced the viscosity of the blood and thereby to have optimized the filtration process and the isolation of individual fetal cells for prenatal diagnosis.

With respect to claim 31,Vona teaches that following pre-amplification, "(f)ive out of 60 µl of the extension product was co-amplified in a final volume of 100ul" using

primers for HLA and p53 (page 60, col. 1; emphasis added). Thus, Vona teaches purifying (increasing the purity of) the pre-amplification product by obtaining 5ul of the pre-amplified DNA (i.e., the extension product) and diluting this extension product in 100ul to allow for the subsequent analysis of the pre-amplified product by PCR analysis. As discussed above, Kalionis teaches analyzing the individual fetal cells to demonstrate that the cells are fetal cells (pages 10 and 18) and to detect a genetic anomaly or to determine the sex of the fetal cells (see pages 9-10 and page 21). Thereby, the combined references teach that the DNA obtained from the individual cell is first pre-amplified, the extension product is diluted in a solution comprising additional PCR components (thereby obtaining a purified preparation of pre-amplified DNA) and amplification is performed to determine the fetal origin and to carry out prenatal diagnosis.

7. Claims 13, 14, 16 and 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kalionis in view of Vona (2000) and Bisconte, and further in view of Bianchi (U.S. Patent No. 5,614,628; cited in the IDS) .

The teachings of Kalionis, Vona and Bisconte are presented above.

With respect to claim 13, the combined references do not teach sequencing the amplified fetal DNA. However, Bianchi (paragraph 31) teaches sequencing amplified fetal DNA in order to detect the presence of genetic variation in the fetal DNA and teaches that sequencing may be used in place of or in addition to detection of genetic variations by PCR or hybridization analysis. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the

method of Kalionis so as to have sequenced the amplified fetal DNA in order to have achieved the benefit of providing a sensitive and effective means for detecting genetic variation in the fetal DNA thereby facilitating the method of prenatal diagnosis.

With respect to claim 14, the combined references do not teach using a probe to analyze the amplified DNA. However, Bianchi (e.g., paragraph 31) teaches that PCR amplified DNA can be analyzed by probe hybridization to detect nucleic acid sequence variations. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kalionis so as to have detected the amplified fetal DNA by probe hybridization in order to have achieved the benefit of providing a sensitive and effective means for detecting genetic variation in the fetal DNA, thereby facilitating the method of prenatal diagnosis.

With respect to claim 16, Kalionis does not specifically teach detecting at least one polymorphism, such a SNP. However, Bianchi teaches methods of prenatal diagnosis which include the detection of polymorphisms, such as that associated with sickle cell anemia (see paragraph 46) and paternally inherited polymorphisms (paragraph 35). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kalionis so as to have specifically detected a polymorphism associated with sickle cell anemia in order to have allowed for the prenatal diagnosis of sickle cell anemia or to have specifically detected the paternally inherited polymorphism disclosed by Bianchi in order to have confirmed the identity of female fetal cells and to have distinguished female fetal cells from maternal cells.

With respect to claim 17, the combined references do not teach analyzing the fetal nucleic acids in order to demonstrate the biparental contribution of fetal DNA.

However, Bianchi teaches methods of prenatal diagnosis wherein the methods are carried out using nucleic acid probes that detect nucleic acids that are specific for both maternally and paternally derived nucleic acid sequences (see, e.g., paragraph 35 and 104-106). In view of the teachings of Bianchi, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kalionis so as to have analyzed the fetal nucleic acids for markers specific for each parent in order to have provided a method that would have allowed one to distinguish between female fetal DNA and maternal DNA, thereby confirming the identity of the fetal cells and which would have allowed for the identification of both paternally and maternally inherited sequences in the fetal cells.

8. Claim 15 is rejected under 35 U.S.C. 103(a) as being unpatentable over Kalionis in view of Vona (2000) and Bisconte, and further in view of Fodor (U.S. Patent No. 6,309,822) .

The teachings of Kalionis, Vona and Bisconte are presented above. The combined references do not teach detecting a genetic anomaly or genotype using DNA probes fixed to a microarray.

However, Fodor teaches methods for detecting mutations and polymorphisms using microarrays wherein a nucleic acid probe comprising a mutation/polymorphism or a wildtype sequence is immobilized onto an array and the array is contacted with a sample nucleic acid (see, e.g., paragraphs 714-716). Fodor (paragraph 368) states that

microarrays can be used to simultaneously analyze multiple samples for a large number of genetic markers and allows for simplified, economized and more generally accessible prenatal screening.

In view of the teachings of Fodor, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kalionis so as to have detected the genetic mutations or polymorphisms using a microarray in order to have obtained the advantages set forth by Fodor of providing a method which allowed for the simultaneous analysis of multiple samples and the detection of a plurality of mutations or polymorphisms, thereby providing a faster, more efficient and economical method of prenatal diagnosis.

9. Claim 18 is rejected under 35 U.S.C. 103(a) as being unpatentable over Kalionis in view of Vona (2000) and Bisconte, and further in view of Pinkel (U.S. Patent No. 6159685).

The teachings of Kalionis, Vona and Bisconte are presented above. In particular, Kalionis teaches prenatal diagnosis of fetal cells by in situ hybridization but does not teach using comparative genomic hybridization (CGH) for prenatal diagnosis.

However, Pinkel (paragraph 41) teaches the method of comparative genomic hybridization and teaches the application of this method to prenatal diagnosis by assaying nucleic acid sequences of fetal cells (see, e.g., paragraphs 8 and 14). Specifically, Pinkel (paragraphs 14 and 41) teaches that CGH employs the methodology of in situ hybridization in order to detect extra or missing copies of whole chromosomes or parts of chromosomes. Pinkel (paragraph 14) states: "(w)hen CGH is applied, for

example, in the fields of tumor cytogenetics and prenatal diagnosis, it provides methods to determine whether there are abnormal copy numbers of nucleic acid sequences anywhere in the genome of a subject tumor cell or fetal cell or the genomes from representative cells from a tumor cell population or from a number of fetal cells, without having to prepare condensed chromosome spreads from those cells. Thus, cytogenetic abnormalities involving abnormal copy numbers of nucleic acid sequences, specifically amplifications and/or deletions, can be found by the methods of this invention in the format of an immediate overview of an entire genome or portions thereof. More specifically, CGH provides methods to compare and map the frequency of nucleic acid sequences from one or more subject genomes or portions thereof in relation to a reference genome. It permits the determination of the relative number of copies of nucleic acid sequences in one or more subject genomes (for example, those of tumor cells) as a function of the location of those sequences in a reference genome (for example, that of a normal human cell)."

In view of the teachings of Pinkel, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kalionis so as to have analyzed the isolated fetal cells by CGH in order to have provided a rapid and effective means for identifying genetic anomalies in the fetal nucleic acid, thereby facilitating the method of prenatal diagnosis.

10. Claim 30 is rejected under 35 U.S.C. 103(a) as being unpatentable over Kalionis in view of Vona (2000) and Bisconte, and further in view of Whatman Cyclopore® Membranes (17 January 2000).

The teachings of Kalionis, Vona and Bisconte are presented above. In particular, Vona teaches isolating large epithelial cells from blood using a polycarbonate track-etch-type filter with calibrated 8 um diameter, cylindrical pores made by Cyclotron technology (page 58, col. 2). Vona does not teach the thickness of the filter and thereby does not teach filters of 12 um.

However, Whatman teaches Cyclopore® membranes that are track-etched and manufactured using Cyclotron technology. It is stated that the polycarbonate filters having a pore size of 0.1 to 12 µm have a thickness of 10 to 20 µm. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have selected an available polycarbonate track-etched membrane manufactured using Cyclotron technology and having an appropriate thickness, including a thickness of 12 µm. Since Vona teaches using the particular polycarbonate track-etched filter made by Cyclotron technology and having a pore size of 8 µm for the isolation of epithelial cells, the ordinary artisan would have selected a similar filter for the isolation of trophoblast cells since trophoblast cells were well known in the art to be epithelial cells of fetal origin. Thereby, the ordinary artisan would have selected one of the well known and available filters, such as the filter of Whatman for the isolation of fetal cells. The thickness of the filter would have been a property inherent to the filter. Moreover, the selection of a filter with the optimum filter thickness would have been well within the skill of the art and obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, in the absence of evidence to the contrary, use of a Whatman

Cyclopore® membrane having a thickness of 12 μm would have been obvious to one of ordinary skill in the art at the time the invention was made.

Regarding the pore density, as discussed above, to have determined the optimum density of the pores that would allow for the isolation and collection of single trophoblast epithelial cells from the filter membrane would have been obvious to one of ordinary skill in the art and well within the skill of the art. As discussed in MPEP2144.05(b), "(w)here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation. In re Aller, 220 F.2d 454, 105 USPQ 233, 235 (CCPA 1955). Again, Bisconte (para [0039] and Vona (page 58, col. 1) each teach the criticality of selecting a filter wherein the pore size is sufficient to retain the cell of interest and wherein the filter allows for the separation and isolation of individual cells. Accordingly, the selection of a polycarbonate filter having an optimum pore density, including a pore density of 1×10^5 pores/cm^2, would have been obvious to one of ordinary skill in the art and well within the skill of the art at the time the invention was made in order to have to have accomplished the objective of isolating and collecting the single fetal cells, thereby facilitating the method of prenatal diagnosis.

11. The art made of record and not relied upon is considered pertinent to applicant's disclosure.

Gentaur BVBV- BIOXYS (available via url: <bioxys.com/i_Whatman/cyclopore_polycarbonate_and_polyester_membranes.htm>) provides detailed information regarding the Whatman Cyclopore® polycarbonate track-

etched membranes. It is disclosed that the membranes having a pore size of 8 μm have a thickness of 12 μm and a pore density of 1×10^5.

RESPONSE TO ARGUMENTS

12. In the response of August 5, 2008, Applicants traversed each of the above rejections. Applicants arguments set forth on pages 10-14 of the response are the same as those presented in the response of October 31, 2007. These arguments were addressed in full in the prior Office action of February 5, 2008 and apply equally to the present arguments and the claims.

At page 14 of the response, Applicants state that the claims have been amended to recite that the filter has a pore size of 8 μm, a pore density of 5×10^4 to 5×10^5 pores/cm^2 , and that a filtration "depressure" of 0.05 to 0.8 bars is applied to the pressure. It is stated that "the inventor has determined which parameters were most critical, which had to be the most carefully chosen, and at which most preferable value ranges these critical parameters should be adjusted. This critical parameters notably include the choice of a filter with a particular pore density, and the application of a particular depressure range."

These arguments have been fully considered but are not persuasive. Applicants do not provide any evidence or scientific arguments to support the conclusion that the claimed pore sizes, density of pores and pressure applied to the filter would not have been obvious. The originally filed specification also does not specifically teach any unexpected results obtained with the claimed parameters and does not provide any

information that would indicate that selection of such parameters would not be obvious or within the skill of the ordinary artisan. Rather, the specification teaches that: "Preferably, the filter has substantially cylindrical pores with a diameter of about 8 μm and a density in the range 5 X 10^4 to 5 X 10^5 pores/cm^2. More preferably, the filter used is graded so that all of the pores have a substantially identical diameter. One example, of a filter that can be used in the method of the invention is a polycarbonate graded filtration membrane of the "Track-Etched Membrane" type with a pore density of 1 x 10^5 pores/cm^2, a thickness of 12 μm and a pore size of 8 μm, such as that sold by Whatman®." There is no evidence of record to indicate that, for example, Applicant tried other filters of different pore densities or other filtration pressures and that such filters and pressures were not effective for isolating the fetal trophoblast cells. Thereby, there is no evidence or cogent arguments to indicate that it was not well within the skill of the art at the time the invention was made to select filters of the appropriate pore density and to have selected the optimum pressure to be applied to the filter.

Moreover, Vona teaches the isolation of epithelial cells using a polycarbonate, track-etched filter made by Cyclotron technology and having a pore size of 8 um. The ordinary artisan would have used a filter similar to that of Vona to isolate the fetal trophoblast cells since it was well known in the art at the time the invention was made that trophoblast cells are epithelial cells of fetal origin. The pore density and thickness of a filter are properties of particular filters that are commercially available. The filter disclosed by Vona appears to be the same as, or at least significantly similar to, the Whatman Cyclopore® membrane filter. As stated in paragraph 10 above, The Whatman

Cyclopore® polycarbonate track-etched membranes is characterized by Gentaur as having a pore size of 8 µm, a thickness of 12 µm and a pore density of 1×10^5. The ordinary artisan would not have been faced with the task of selecting from an overwhelming number of possible filters, as suggested by Applicants. Rather, the ordinary artisan would have been motivated to have used the same or a similar filter used by Vona for isolating epithelial cells from blood. To have selected such a filter from those commercially available, having the same properties disclosed by Vona, would have been obvious to and within the skill of one of ordinary skill in the art. Again, Applicants do not provide any evidence to establish any unexpected results obtained with the claimed filters, in order to support Applicants conclusion that the selection of such filters would have been unobvious and not within the skill of the ordinary artisan.

The response states that Vona does not teach the criticality of selecting a filter with the pores spaced apart to allow for separation and collection of cells. This argument has also been fully considered but is not persuasive. Vona teaches that the purpose of the method is to isolate the rare epithelial target cells so that the epithelial cells are trapped onto the filter and the smaller cells present in blood pass through the filter (page 58, col. 1). Vona teaches that the cells retained on the filter are then analyzed for their morphology by hematoxylin-eosin staining or immunostaining (page 59, col. 1). Further, Vona teaches that the method requires laser microdissection of individual cells formed on hematoxylin-eosin stained filters, wherein single spots are cut from the filter and individual cells are dissected with a laser microbeam (page 59, col. 2). Vona also teaches "isolation of trophoblastic cells from the peripheral blood of

pregnant woman" in order to improve prenatal diagnosis of genetic diseases (page 62, col. 2). Thereby, the ordinary artisan would have recognized that the filter should be selected so that the pore size was sufficient to isolate and separate fetal trophoblastic cells from other cells present in maternal blood. Further, the ordinary artisan would have recognized that the density of the pores should be selected so that individual fetal trophoblast cells could be analyzed by morphological staining and immunostaining techniques, and so that individual cells could be dissected from the filter with a laser microbeam. Clearly, if the pores were not sufficiently separated from one another at an appropriate density, then the method could not achieve its stated outcome of isolating individual fetal cells retained on the filter.

The response states that "(i)t is respectfully submitted that the complexity of the problem addressed by the presently claimed invention has been underestimated by the Examiner. The problem solved by the present invention does not simply amount to the separation and collection of individual cells; rather, it relates, inter alia, to the isolation of very rare circulating cells present in a very complex mixture of cells." This argument is not convincing because Vona solves this same problem. Vona teaches the ISET/RT-PCR method can be applied to the isolation of trophoblastic cells for prenatal diagnosis (page 62, col. 2). Moreover, Vona clearly teaches that the ISET method is one for the isolation of rare cells in a collection of complex cells. For instance, Vona states "(w)e have developed a new assay, ISET (isolation by size of epithelial tumor cells, which allows the counting and the immunomorphological and molecular characterization of circulating tumor cells in patients with carcinoma, using peripheral blood sample

volumes as small as 1 ml" (see abstract). Applicants' response does not provide any evidence or scientific arguments as to why the teachings of the combined references would not have been sufficient to have reasonably enabled the application of the method of Vona to the isolation of fetal cells from maternal blood. That is, Applicants have not established why the isolation of fetal trophoblast cells from maternal blood is of a more complex nature than the isolation of epithelial tumor cells from blood. For example, what additional cells are present in maternal blood (as compared to peripheral blood of a subject with cancer) that would hinder the isolation of trophoblast cells from maternal blood?

The response states that "a vacuum pump (as it is disclosed in the Vona reference) is not capable of apply a depressure of 0.05-0.8 bars." This argument is not persuasive because Applicants do not provide any evidence or scientific arguments to support this allegation. As set forth in MPEP 2145, the arguments of counsel cannot take the place of evidence in the record. Further, in view of the amendment to the claims to recite that the method includes applying a pressure of 0.05 to 0.8 bars, Bisconte has been cited as teaching methods of isolating rare epithelial cells from blood using a 0.8 um filter, wherein a pressure of 0.5 bars is applied to the filter.

At page 18, Applicants state that "(a)s a matter of fact, the identification of the criticality of these parameters was not immediate to even the present inventor of the presently claimed invention, when she tried to achieve the isolation of the very rare circulating fetal cells. None of the cited references disclose or suggest that the pore density of the filter and the depressure value would be a critical issue to address the

problem of the isolation of the very rare circulating fetal cells. To the contrary, the Kalionis reference suggests to one of ordinary skill in the art that these two parameters are of no importance." These arguments have also been fully considered but are not persuasive. Applicants do not cite a specific teaching in Kalionis which indicates that the authors therein concluded that pore density of the filter and pressure applied to the filter were "of no importance." Further, the arguments are not persuasive because Applicants themselves have not established that it would not have been within the skill of the art to have selected the optimum parameters for filtration. Stating the conclusion that the parameters are critical is not equivalent to providing scientific arguments or evidence to establish why one of ordinary skill in the art would not have had a reasonable expectation of success of applying the filtration method of Vona to the isolation of fetal cells from maternal blood. Again, the prior art clearly provides the motivation to use the filtration method to isolate individual fetal trophoblast (epithelial) cells from maternal blood and teaches that the objective of the method is to obtain cells that can be stained or immunologically stained for analysis without damaging the cells (page 60, col. 2) and to retain the epithelial cells on the filter so that the cells could be individually dissected and isolated from the filter. Thus, the ordinary artisan would have clearly recognized that pore size, pore density and applied filtration pressure would affect the ability to achieve the stated outcome of separating fetal cells in an undamaged state and isolating individual fetal cells from the filter. In the absence of evidence to the contrary, it is maintained that it would have been obvious to one of ordinary skill in the art and well within the skill of the art to have selected the optimum filtration conditions, including the

parameters of an 8 µm filter, a pore density in the range 5 X 10^4 to 5 X 10^5 pores/cm^2, and an applied pressure of 0.5 bars, for the isolation of fetal cells from maternal blood for the purposes of prenatal diagnosis of the fetal cells.

Regarding Applicants arguments at pages 9-14 of the response, those arguments as previously addressed in the Office action of February 5, 2008, are again addressed below:

Applicants state Kalionis does not teach step c) of collecting an individual cell from a filter. This argument has been fully considered but is not persuasive because the rejection is not made over the Kalionis reference alone. One cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In the present situation, the rejection is based on the combined teachings of Kalionis and Vona. It is acknowledged that while Kalionis teaches analyzing the individual cells isolated on the filter to confirm the identity of the fetal cell and to carry out prenatal diagnosis, Kalionis does not teach collecting the individual cells. However, as discussed in the above rejection, Vona teaches a method of ISET wherein individual cells trapped on a filter are collected and further analyzed by PCR. Vona (page 62) specifically teaches applying this technology to fetal cells present in maternal blood: "(t)he potential uses for ISET go well beyond the field of oncology, because it allows the isolation of large, circulating, nontumorous cells. For example, the isolation of trophoblastic cells from the peripheral blood of pregnant women has been initiated in our laboratory and

may constitute an important step toward improving the prenatal diagnosis of genetic diseases."

The response asserts that "the method of Kalionis is suitable for late stage gestation (7 1/2 months of gestation), but not for early stage, contrary to the claimed invention (5 weeks of gestation), which implies that the method of Kalionis is not enabled for pre-natal diagnosis." This argument has also been fully considered but is not persuasive because again the rejection is not made over the teachings of Kalionis alone but rather is based on the combined teachings of Kalionis and Vona. Further, Applicants have not provided any evidence or scientific arguments to establish that the method of Kalionis in view of Vona cannot be applied to prenatal testing. Similarly, Applicants argument that the method of Kalionis can only be applied at 7 ½ months gestation is not supported by any factual scientific arguments or evidence. In fact, Applicants assertion is contradictory to the teachings of Kalionis wherein it is stated that the disclosed method of isolating trophoblasts from maternal blood is applicable to prenatal diagnosis. For example, Kalionis (page 1) states "(t)his invention relates to a method for the isolation of fetal cells, and in particular trophoblast (placental) cells, from the peripheral blood of a pregnant mammal, especially a pregnant human. The isolation of these cells from maternal blood enables genetic and/or biochemical information about the fetus to be obtained." Lastly, this argument is not persuasive because the present claims do not require diagnosis prior to 7 1/2 months gestation and thereby Applicants are arguing limitations that are not in the claims. Only claim 20 is directed to the time of gestation at which the analysis occurs. Claim 20 encompasses the analysis of a blood

sample obtained after the fifth week of pregnancy and thereby also encompasses analyzing blood on or after the 7 1/2 month of gestation. Thereby, the claims do not in fact require analyzing blood at 5 weeks of gestation.

The response asserts that Kalionis does not address the problem of prenatal diagnosis. This argument is not convincing because as stated above Kalionis clearly teaches that the method is one to analyze the genetic information of the unborn fetus, and thereby constitutes a method of prenatal diagnosis. See also page 5 of Kalionis wherein it is stated that "(b)y carrying out in situ hybridization with probes specific for trophoblast mRNA and with probes specific for human chromosomes in the nucleus of trophoblast cell, it is possible to obtain information on the chromosomal complement of the fetus and thereby carry out prenatal diagnosis" (emphasis added).

Applicants assert that it is not obvious to combine references unless there is a specific advantage gained from the modification. This argument is not convincing because Vona specifically teaches the modification – i.e., applying ISET to the isolation of fetal trophoblast cells from maternal blood - and teaches the advantage of the modification - i.e., to provide a rapid and effective means to isolate an individual trophoblast cell from maternal blood, wherein the genetic material of the isolated cell can be further analyzed by the highly sensitive method of primer-extension preamplification (PEP) PCR. As stated by Vona (page 61, col.2), the method of ISET is easy to perform, rapid and inexpensive and allows for the isolation of a specific cell type in a population of different cell types.

Applicants assert that in the method of Vona "there is no cell isolation step at all." This argument is not persuasive because Vona is clearly directed to a method of laser microdissection for isolating individual cells - i.e., ISET - and Vona specifically teaches application of ISET to fetal trophoblast cells present in maternal blood (page 62, col. 2). It is stated that in the method of Vona, only tumor cells are retained on the filter, whereas in the present invention a complex mixture of cells are retained on the filter. However, in the method of Vona the tumor cells present in peripheral blood are treated by filtration. In the present invention, fetal cells present in maternal blood are filtration. Applicants have not established what complex mixture of cells are present in maternal blood and are absent in blood of patients with cancer.

Applicants assert that trophoblast cells in maternal blood are quite rare and that there is nothing in Vona to suggest that fetal cells in a complex population of maternal cells can be isolated. This argument is not convincing because Vona in fact specifically teaches the application of ISET to the isolation of individual cells in a population of complex cells. For example, Vona teaches that ISET is used to analyze circulating tumor cells in peripheral blood samples from patients with carcinoma (see abstract and page 61). Further, Vona (page 62, col. 2) specifically teaches that their laboratory has begun to study fetal trophoblast cells isolated from maternal blood using the method of ISET. Vona also teaches that the size of the filter allows one to separate larger cells from other cells present in a complex sample, such as large epithelial cells from peripheral blood leukocytes, thereby allowing for the isolation of individual cells in a complex population of cells (page 58, col. 1). Additionally, Kalionis teaches that there

are a sufficient number of trophoblast cells present in maternal blood to allow for the enrichment and analysis of individual trophoblast cells (e.g., pages 2-3). Kalionis (page 3) states that the filtration method disclosed therein is "a method for easily enriching and identifying trophoblast cells in maternal blood in the presence of a population of blood cell types." Obviousness does not require absolute predictability but only the reasonable expectation of success. See In re Merck and Company Inc., 800 F. 2d 1091, 231 USPQ 375 (Fed. Cir. 1986) and In re O'Farrell, 7 USPQ2d 1673 (Fed. Cir. 1988). In the present situation, the teachings of Kalionis and Vona provide more than a reasonable expectation of success of applying the ISET method to the isolation and PEP PCR analysis of trophoblast cells in maternal blood.

The response states that Vona fails to disclose a method wherein the amplification step would comprise the demonstration of "two clinical features," as is recited in step f). This argument is not persuasive since the claims are not generically directed to a method wherein the amplification step demonstrates two clinical features. Further, the rejection is not made over Vona alone, but rather over the combination of Vona and Kalionis. Thereby, Applicants cannot establish the non-obviousness of the claimed invention by addressing the teachings of Vona and Kalionis separately. Lastly, Vona does in fact teach that the isolated cells are further analyzed by PEP PCR and exemplifies methods wherein two distinct amplification products are produced - p53 and HLA amplification products, thereby allowing for the analysis of "two clinical features." Moreover, Kalionis was specifically cited for its teachings of obtaining an individual trophoblast cell from maternal blood, analyzing the trophoblast cell to ensure its fetal

origin and analyzing the fetal cell for genetic abnormalities. Thereby, in combination, the cited references teach a method wherein the trophoblast cells isolated from maternal blood are further subjected to preamplification and the preamplification products are analyzed by PCR to verify the fetal origin of the isolated single cells and to carry out prenatal diagnosis.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carla Myers whose telephone number is (571) 272-0747. The examiner can normally be reached on Monday-Thursday from 6:30 AM-5:00 PM. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla, can be reached on (571)-272-0735.

The fax phone number for the organization where this application or proceeding is assigned is (571)-273-8300.

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